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July 16, 2011

VIA EMAIL

Ms. Monet Vela
Office of Environmental Health Hazard Assessment
P.O. Box 4010, MS-23B
Sacramento, California 95812-4010
P65Public.Comments@oehha.ca.gov

Dear Ms. Vela:

On behalf of Ferro Corporation, we are submitting the enclosed comments on OEHHA's proposed Maximum Allowable Dose Level (MADL) for butyl benzyl phthalate (BBP). Ferro is a major producer of BBP.

Ferro appreciates OEHHA's efforts to promulgate a MADL, which will be helpful to persons in determining whether a Proposition 65 warning is required for their product. The proposed MADL is 1,200 ug/day, based on a NOEL of 20 mg/kg/day. For the reasons given in these comments, Ferro believes that 50 mg/kg/day is a very conservative NOEL for BBP's developmental toxicity, and we ask OEHHA to adopt that NOEL for its calculation of the MADL. It would yield a MADL of 2,900 ug/day.

If OEHHA has any questions or wishes additional information, please contact the undersigned at 202-637-2229 or ann.claassen@lw.com, or Bill Rawson at 202-637-2230 or william.rawson@lw.com.

Sincerely,

William K. Rawson
Ann Claassen
of LATHAM & WATKINS LLP

Enclosure

cc: Alan Olson, Ferro Corporation

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

PROPOSAL TO ADOPT A MAXIMUM ALLOWABLE DOSE LEVEL (MADL) for BUTYL BENZYL PHTHALATE (ORAL EXPOSURE)

Pursuant to

SAFE DRINKING WATER AND TOXIC ENFORCEMENT ACT OF 1986 (PROPOSITION 65)

COMMENTS OF FERRO CORPORATION

July 16, 2012

EXECUTIVE SUMMARY

Ferro Corporation (Ferro) is submitting these comments to the California Office of Environmental Health Assessment (OEHHA) regarding its proposed Maximum Acceptable Dose Level (MADL) for butyl benzyl phthalate (BBP). Ferro is a major manufacturer of BBP.

Ferro expresses its appreciation to OEHHA for its efforts to develop a MADL for BBP. OEHHA is proposing to set a MADL for BBP of 1200 micrograms per day (ug/day), derived from a no observed effect level (NOEL) of 20 milligrams per kilogram per day (mg/kg/day) from Nagao et al. (2000). Ferro believes that this MADL is overly conservative and that the science clearly supports a MADL of 2900 ug/day based on a NOEL of 50 mg/kg/day from the Tyl et al. (2004) study.

Nagao et al. (2000) was a two-generation reproductive toxicity study. The NOEL of 20 mg/kg/day is based on reduced pup weights on post-natal day (PND) 0, for the mid-dose of the F1 generation – 100 mg/kg/day. Those effects reversed and there was no effect on survival or development of the pup. There was no effect at that dose in the F2 generation, which had a litter size comparable to controls. Therefore, the pup weight effect well may be due to litter size rather than treatment, such that 100 mg/kg/day could be considered a no observed adverse effect level (NOAEL). Thus 20 mg/kg/day is a very conservative NOEL.

Tyl et al. (2004) also was a two-generation reproductive toxicity study. The NOEL of 50 mg/kg/day for it is based on reduced anogenital distance (AGD) in male pups at the next highest dose – 250 mg/kg/day. The authors considered 250 mg/kg/day to be a NOAEL because there were no effects on reproductive development, structures, or functions at that dose. Thus, 50 mg/kg/day is a very conservative NOEL for this study.

OEHHA has proposed 20 mg/kg/day as the NOEL because Sumner et al. (2009) reported effects on sensitive indicators of male reproductive development (retained areolae and reduced AGD) at 25 mg/kg/day. However, that study was not designed for risk assessment purposes. It was a hypothesis-generating study to explore use of metabolomics as biomarkers for BBP exposure, not a study designed to identify a NOEL. The study used only 3 dams per sex per dose and only two doses besides the control, with a very wide range between the doses. The authors acknowledged that interpretation of their observations was limited due to the small number of animals and they neither ran statistics on the reproductive toxicity parameters nor identified a NOEL or lowest observed effect level (LOEL).

OEHHA ran statistics on the Sumner et al. data on a per-pup basis and report that the increase in retained areolae on PND 11 was statistically significant. However, the appropriate unit for statistics for a developmental study is the litter, not the individual pups. Furthermore, the effects observed in the male pups were fully reversed by PND 26 and no effect on reproductive function or structure was reported. Therefore it is questionable that the effects were adverse.

Given the existence of two robust two-generation studies, and the limitations of the Sumner et al. study for risk characterization, it is inappropriate to use the Sumner et al. study for developmental of the MADL. Both Nagao and Tyl met the guideline standard of three doses

plus control. Both exceeded guideline requirements for number of animals – 25 and 30 animals per sex per dose for the F0 generation. Together they provide a conservative NOEL of 50 mg/kg/day.

The conservatism of this NOEL is further supported by recent conclusions from leading researchers of male reproductive toxicity, including effects of phthalates, that the effects seen in the rat studies (Nagao, Tyl and Sumner) are species-specific, and that humans are more refractory to such effects. Thus, the weight of the evidence is that 50 mg/kg/day as a NOEL, yielding a MADL of 2900 ug/day, is highly conservative.

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INTRODUCTION

Ferro Corporation (Ferro) is submitting these comments to the California Office of Environmental Health Assessment (OEHHA) regarding its proposed Maximum Acceptable Dose Level (MADL) for butyl benzyl phthalate (BBP).[1] Ferro Corporation is a major manufacturer of BBP.

First, Ferro expresses its appreciation to OEHHA for its efforts to develop a MADL for BBP. The promulgation by the State of a "safe harbor" provides greater certainty to persons trying to determine whether a Proposition 65 warning is required for their products.

OEHHA is proposing to set a MADL for BBP of 1200 micrograms per day (ug/day), derived from a no observed effect level (NOEL) of 20 milligrams per kilogram per day (mg/kg/day) from Nagao et al. (2000). Ferro believes that this MADL is overly conservative and that the science clearly supports a MADL of 2900 ug/day based on a NOEL of 50 mg/kg/day from the Tyl et al. (2004) study. OEHHA's reliance on Sumner et al. (2009) to justify selection of the lower NOEL is not warranted given the nature of that study and the nature of the observed effects, as explained below.

Robust Reproductive Toxicity Studies Indicate that a Conservative NOEL for BBP is 50 mg/kg/day

As acknowledged by OEHHA, there are two well-conducted, two-generation reproductive studies available for BBP – Nagao et al. (2000) and Tyl et al. (2004). Nagao et al. used 25 dams per dose; Tyl et al. used 30 dams per dose. The two lowest treatment doses of Nagao – 20 and 100 mg/kg/day – bracket the lowest treatment dose used in Tyl – 50 mg/kg/day. In both Nagao and Tyl, no adverse effects at all were seen at the lowest dose (20 and 50 mg/kg/day, respectively). Between the two studies, the next highest dose was 100 mg/kg/day. Thus, overall the NOEL for BBP from these two studies is 50 mg/kg/day.

With regard to these two studies, a NOEL of 50 mg/kg/day is in fact quite conservative. It is arguable that 100 mg/kg/day in Nagao and 250 mg/kg/day in Tyl should be considered the respective NOAELs for those studies. In Nagao, the effect was reversible and explainable by the specific circumstances of that study. In Tyl, the effect was considered non-adverse by the authors because there were no effects on reproductive function, development or structure.

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[1] OEHHA, Notice of Proposed Rulemaking, Title 27, California Code of Regulations, Amendment to Section 25805 Specific Regulatory Levels: Chemicals Causing Reproductive Toxicity, Butyl Benzyl Phthalate (oral exposure) (June 1, 2012), http://www.oehha.ca.gov/prop65/law/060112bbpnotice.html.

For the Nagao Study, the NOAEL Arguably Was 100 mg/kg/day, because the Effect at that Level Was Reversible and Was Plausibly Due to Litter Size rather than BBP

The effect seen at the Nagao "LOEL" of 100 mg/kg/day could well be an artifact of the unusually large litter size at that dose. To explain: The effect observed at 100 mg/kg/day in Nagao was reduced pup weight at post-natal day (PND) 0 in the F1 generation. A close review of the data reveals that the number of pups per litter at that dose was substantially larger than in the control and in each other treatment group, and this could well account for their lower weight at PND 0. That this is highly plausible is indicated by the facts that: 1) at PND 4, when the pups were culled to have equal numbers of pups per litter, and onward, there was no statistical difference between the 100 mg/kg/day group and the controls; and, 2) in the F2 generation, where there was not a difference in litter size between the 100 mg/kg/day group and the controls, there also was no effect on weight at PND 0 (or at any other day).

As stated by EPA in its guidelines for reproductive toxicity testing:

> *Individual pups in large litters tend to be smaller than pups in smaller litters. Thus, reduced birth weights that can be attributed to large litter size should not be considered an adverse effect unless the increased litter size is treatment related and the subsequent ability of the offspring to survive or develop is compromised.*

(EPA, 1996, Section II.B.2.a.)

Chahoud and Paumgarten (2009) examined litters from 241 untreated rats and found an inverse relationship between litter size and pup post-natal body weight gain. According to the authors, the inverse relationship, i.e., decreased pup body weight with increasing litter size, is present during the entire lactation period and manifests as early as the day of birth (PND 0). They also point out that litter size affects the day of attainment of some milestones of somatic maturation. The authors cite a number of previous studies supporting their observations.

Reduction in pup body weight (in untreated animals) is associated with limited availability of maternal milk (Chahoud and Paumgartten, 2009). Although milk volume increases with litter size, milk production does not increase above litter sizes of 11 pups (Chahoud and Paumgarten, 2009). Nagao reports 14.9 pups born alive per litter in the 100 mg/kg/day group at PND 0 versus 13.1 in the control group.

Thus, the evidence in Nagao indicates that the reduced pup weights at PND 0 for the F1 100 mg/kg/day group can indeed be attributed to large litter size. Because of the lack of any such effect in the F2 generation at that dose, it appears the increased litter size is not treatment related. The subsequent ability of the F1 offspring to survive and develop was not compromised. Therefore, selection of 20 mg/kg/day as the NOEL for Nagao et al. (2004) is quite conservative. EPA's guidelines would indicate that the appropriate NOAEL for that study is 100 mg/kg/day.

For the Tyl Study, the NOAEL Arguably Was 250 mg/kg/day, because the Effect Observed at that Level Had No Adverse Consequences for the Reproductive Function of the Animals

In the Tyl study, the next highest dose above the NOEL of 50 mg/kg/day was 250 mg/kg/day. At that dose, the effect observed was a reduction in anogenital distance (AGD) in the male pups at PND 0. There was no effect at that level on other sensitive indicators of reproductive toxicity, e.g., no effect on retained nipples or areolae, sperm parameters or body weight. The authors considered 250 mg/kg/day to be a NOAEL, because reproductive function of the animals was not impaired, and there was no effect on reproductive development or structure.

Thus, from the Nagao and Tyl studies, 50 mg/kg/day is clearly a conservative NOEL.

The Sumner et al. Study Does Not Provide a Basis for Selecting the Nagao NOEL over the Tyl NOEL

In its Initial Statement of Reasons for the proposed BBP MADL, OEHHA used the Sumner et al. (2009) mechanistic study as the basis for selecting the Nagao NOEL of 20 mg/kg/day.[2] This is inappropriate because the Sumner study was a hypothesis-testing study rather than a study designed to characterize risk for risk management purposes. It used a small number of animals and a non-orthodox (for a risk assessment study) dose range. These are not criticisms of the study itself, which appears to have been well-conducted; the criticism is for using the study in a risk assessment context. OEHHA has proposed to do so by using inappropriate statistics and by ignoring the weight of the evidence that the developmental toxicity NOEL for BBP is at least 50 mg/kg/day.

It is Inappropriate to Use a Hypothesis-Testing Study for Regulatory Risk Management when Robust Risk Assessment Studies are Available

The Sumner authors describe the purpose and results of their study thus:

> *This study was conducted to determine the utility of metabolomics in providing biomarkers in non-invasive biological fluids for the study of reproductive and developmental toxicology, and providing insights regarding mechanisms in generation of adverse effects.*
>
> ...
>
> *In summary, the study has demonstrated the use of urinary metabolites in a classification approach of prior exposure (to include prior* in uteri *exposure) past the time of the presence of the parent compound and metabolites derived from the parent compound. This approach may find important application in the assessment of prior exposure to*

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[2] OEHHA, Initial Statement of Reasons, Title 27, California Code of Regulations Proposed Amendment to Section 25805(b), Specific Regulatory Levels: Chemicals Causing Reproductive Toxicity, Butyl Benzyl Phthalate (Oral Exposure) (undated), p. 7 of 12.

classifications of environmental toxicants or drugs, and through mapping to metabolic pathways can provide important mechanistic insights.

(Sumner et al., 2009, pp. 712 and 713.)

In other words, this study was designed to explore the viability of using metabolomics to assess prior exposure, and showed that this method worked with BBP. It was not designed to determine a NOEL for purposes of deriving a health benchmark such as a MADL.

In contrast, Nagao and Tyl both *were* designed for risk assessment. The conclusion of the Nagao study is:

From these data, it would appear that 20 mg/kg BBP is a no observed adverse effect level (NOAEL) for reproductive effects on parent rats and the next generation.

(Nagao et al., 2000, p. 530.)

The conclusion of the Tyl study is:

Therefore, in rats, under the conditions of this study:

- *The F0 and F1 parental systemic and reproductive NOAEL is 3750 pap (equivalent to approximately 250 mg/kg per day).*
- *The F1 and F2 offspring reproductive toxicity NOAEL also is 3750 pap (equivalent to approximately 250 mg/kg per day).*
- *The F1 and F2 offspring reproductive toxicity NOEL (no observable effect level) in males is 750 pap (equivalent to approximately 50 mg/kg per day), based on the significantly shortened anogenital distance in F1 and F2 male pups at birth at 3750 (and at 11,250) pap, with no effects on reproductive development, structures, or functions at 3750 mg/kg per day.*

(Tyl et al., 2004, p. 262.)

That is, the ultimate purpose of these studies was to identify a NOAEL. The two studies did so by following guidelines for number of animals and doses.

EPA test guidelines specify 20 males and enough females to give 20 pregnancies at each dose (EPA, 1996, Section II.A.4; EPA, 1998, p. 2). Similarly large numbers of animals are specified by other authorities and consensus bodies (FDA, 2000a; OECD, 2001; European Medicines Agency, 1994). Nagao began with 25 animals per sex per dose, Tyl with 30 animals per sex per dose. The studies also were conducted otherwise in accordance with consensus guidelines for two-generation reproductive toxicity studies – with enhancements for detecting sensitive indicators of reproductive toxicity – and so are appropriately used for risk assessment.

Even for a reproductive/developmental toxicity screen, EPA recommends starting with at least 10 animals per sex per dose, and states that 8 pregnant females per group "is the minimum acceptable number of pregnant females per group" (EPA, 2000, p. 3). In addition, for both a

screen and a multi-generation reproductive toxicity test, the guidelines call for at least three dosage levels and a control (id; EPA, 1998, p. 2; EPA, 1996, Section III.A.4.) Moreover, both the current EPA Health Effect Test Guidelines for Reproductive and Fertility Effects Testing and the OECD Guidelines for Reproductive Toxicity Testing state that two- or four-fold intervals are frequently optimal for spacing dose levels and adding an additional dose group is preferable to using "very large intervals (e.g., more than a factor of 10) between dosages" (EPA, 1998, p. 2; OECD, 2001, Paragraph 13).

The Sumner study used only 3 dams per dose group, the doses being 0, 25 and 750 mg/kg/day – a 30-fold interval between treatment levels. Thus, the Sumner et al. study, with a small number of pregnant females per dose and only two doses beside the control spaced more than 10-fold apart, does not meet guidelines even for a reproductive/developmental toxicity screening test and certainly not for a more definitive test meant to support risk characterization of a chemical. Again, this is not a criticism of the Sumner study per se, which was designed for a different purpose; rather, it is a criticism of the study being used for establishment of a risk level such as the MADL.

Given the existence of two robust, guideline studies designed to characterize the risks of BBP and to identify a NOEL, it is inappropriate to use the Sumner study – which was not designed for risk characterization or NOEL identification – to identify the NOEL for BBP.

The Appropriate Statistical Unit for a Reproductive/Developmental Toxicity Study is the Litter

Sumner et al. did not conduct statistical analyses of their data, nor did they identify a NOEL or LOEL. This is appropriate given the small number of animals per dose. In fact, the authors acknowledge that "interpretations of this study are limited due to the small study size" (Sumner et al., 2009, p. 712).

OEHHA nevertheless conducted statistical analyses using the reported data and concluded that retained areolae in male pups were statistically significant at 25 and 750 mg/kg/day, *on an individual pup basis*. This is inappropriate not only because of the small size of the study, but also because the proper statistical unit for a reproductive/developmental toxicity study is the litter, not the individual pup. EPA's guidelines for developmental toxicity testing state:

> *Because the maternal animal, and not the conceptus, is the individual tested during gestation, data generally are calculated as incidence per litter or as number and percent of litters with particular endpoints.*
>
> (EPA, 1991, Section 3.1.1.2.)

The rationale for using the litter as the unit is further highlighted in the following discussion relating to statistics for an epidemiological study in EPA's reproductive toxicity risk assessment guidelines:

As in studies of test animals, pregnancies experienced by the same woman are not fully independent events. For example, women who have had fetal loss are reported to be more likely to have subsequent losses (Leridon, 1977). In test animal studies, the litter can be used as the unit of measure to deal with no independence of response within the litter. In studies of humans, pregnancies are sequential, requiring analyses which consider no independence of events (Epidemiology Workgroup for the Interagency Regulatory Liaison Group, 1981; Kissling, 1981; Selevan, 1981; Zeger and Liang, 1986). If more than one pregnancy per woman is included, as is often necessary with small study groups, the use of nonindependent observations overestimates the true size of the groups being compared, thus artificially increasing the probability of reaching statistical significance (Stiratelli et al., 1984).

(EPA, 1996, Section III.C.1.e, emphasis added.)

The given in this passage is that, in an animal study, the litter should be used as the unit of measure, because effects on pups from the same litter are not fully independent events.

FDA requires that developmental toxicity results be reported on a per litter and not a per pup basis, because it is the dam that is treated, not the individual developing pups. The agency's guidelines for developmental toxicity testing state the following:

D. Reporting the Results of Developmental Toxicity Studies

Reports of all studies should contain the information required by the Good Laboratory Practice Regulations, including a copy of the study protocol and all amendments, absolute values for all parameters, complete data (individual pups) and tables of data summarized and analyzed by litter.[3] *Because the maternal animal is treated during gestation rather than the developing organism, data should be calculated as incidence per litter or as number and percent of litters with particular end points. The dosage rate of the test substance (doses) should be reported as mg/kg/day (milligrams of test substance per kilogram of body weight per day).*

Problems commonly encountered in the review of developmental toxicity studies include insufficient numbers of pregnant animals per control or treatment group, non-random selection procedures, and statistical analyses of data on a per-fetus basis instead of a per-litter basis. Careful consideration of recommended guidelines and a review of protocols by the Agency before studies are conducted should help eliminate such problems.

(FDA, 2000b, emphasis added.)

Thus, it is inappropriate to use the Sumner data to select a NOEL not only because the study was not designed for such use, but also because it is inappropriate to use per-pup statistics to identify a significant effect. OEHHA should follow the lead of the Sumner authors and not use their data as a basis for identifying a NOEL or LOEL.

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3 We note that these data were reported by Tyl et al. (2004).

The Effects at 25 mg/kg/day in Sumner et al. Should Not Be Considered Adverse

Sumner et al. reported two types of reproductive effects at 25 mg/kg/day. One was observation of retained areolae in 7 of 16 males at PND 11, but these effects were fully reversible – at PND 26 there were no retained areolae. The other reported effect was reduced AGD in 2 of the 16 males at PND 21, but not at PND 0 nor PND 26. Thus, these effects also were fully reversible. There was no evidence of adverse effect on the development of the male reproductive tract nor on testosterone or leuteinizing hormone levels at 25 mg/kg/day. Therefore, Ferro believes it is improper to consider the effects seen at 25 mg/kg/day as adverse developmental effects. Rather, to the extent the study is used at all to define risk levels, the low dose should be considered a NOAEL, which is congruent with the NOELs from Nagao and Tyl.

The Weight of the Evidence Is That a Conservative NOEL for BBP Is 50 mg/kg/day

OEHHA's regulations for determining a MADL state that the determination of the MADL "shall be based on evidence and standards of comparable scientific validity to the evidence and standards which form the scientific basis for the listing of a chemical as known to the state to cause reproductive toxicity." 27 CCR § 25801(a). OEHHA's guidelines for listing a chemical as known to the state to cause reproductive toxicity state: "In evaluating the sufficiency of data, a "weight-of-evidence" approach shall be used to evaluate the body of information available for a given chemical" (OEHHA, 1993, Section I.D). A weight of the evidence approach regarding the development toxicity of BBP demonstrates that 50 mg/kg/day is a conservative NOEL.

As discussed above, the appropriate NOAEL for Nagao is 100 mg/kg/day, because the effect seen at that level can be attributed to litter size effect rather than treatment. Further, the effect was fully reversible and the animals showed no decrement in reproductive development or function. Similarly, the appropriate NOAEL for the Tyl study arguably is 250 mg/kg/day. The effect at that dose level was reduced AGD at PND 0. There was no effect on reproductive development, structure, or function at that dose, causing the authors to consider it a NOAEL. Thus, these data arguably support a NOAEL of 100 mg/kg/day. This is consistent with a study by Piersma et al. (2000), who used benchmark dose techniques to estimate 95 mg/kg/day as the dose associated with a 1% increase in abnormal testis location, the most sensitive indicator of the development of the male reproductive tract. Thus, these three studies indicate that 50 mg/kg/day is a conservative NOEL for BBP's developmental toxicity.

The only data that provide a basis for considering 20 mg/kg/day to be the NOEL are the data from Sumner et al. However, a review of the data from that study and from Nagao and Tyl is highly suggestive that the observations at the low dose in Sumner were a statistical anomaly, rather than an indication of treatment-related effects, for the following reasons.

At 25 mg/kg/day in Sumner, 7 of 16 male pups had retained areolae at PND 11 (but not at PND 26). In contrast, in Tyl, at 50 mg/kg/day – twice the dose of the Sumner low dose – none of about 173 F1 male pups and none of about 201 F2 male pups exhibited retained areolae at PND

11-13.[4] At 250 mg/kg/day – ten times the low dose of Sumner – 1 or 2 of about 191 F1 male pups and about 10 of 177 F2 male pups had retained areolae at PND 11-13. Of note, the control incidences were about 5 of 178 F1 males, and about 4 of 206 F2 males. Thus, at 50 mg/kg/day, the incidence of retained areolae was lower than that of controls, and the incidence at 250 mg/kg/day was not statistically greater than controls.

The other reproductive effect reported for 25 mg/kg/day in Sumner was 2 of 16 male pups with reduced AGD. Tyl and Nagao do not report numbers of individual pups with reduced AGD. However, there was no statistical difference in AGD between controls and about 374 male pups (F1 and F2) at 50 mg/kg/day in Tyl. In Nagao, there was no statistical difference in AGD between controls and 125 male pups at 20 mg/kg and 181 male pups at 100 mg/kg/day.

These numbers are summarized in Tables 1 and 2. Stated another way, weighing against 7 of 16 pups in Sumner with retained areolae at 25 mg/kg/day are 11-12 of 742 pups in Tyl at doses 2 to 10 times higher. Further, across all three studies, there was no statistical reduction in AGD at doses up to 100 mg/kg/day. Given this, Ferro believes the weight of the evidence clearly is that 50 mg/kg/day is a conservative NOEL for the developmental toxicity of BBP.

Recent Data Indicate that the Developmental Effects in Rats Treated with BBP Are Species-Dependent

In addition to the foregoing evidence that 50 mg/kg/day is a very conservative NOEL for developmental toxicity in rats treated with BBP, there are recent data from two separate laboratories indicating that those effects are specific to the rat and not relevant to humans. These data provide further evidence that a NOEL of 50 mg/kg/day is a very conservative basis for establishing a MADL for BBP.

In an elegant series of xenograft experiments using mouse, rat and human fetal testis Hegel, et al. (2012) showed that, when the host rats or mice were dosed with dibutyl phthalate (which has a metabolite in common with BBP), only rats exhibited suppressed steroidogenesis in fetal testicular tissue regardless of the host for gestation. Human fetal testis xenografted into rats and mice gavaged with phthalate did not show alteration in steroidogenesis.

A group led by Richard Sharpe showed that Chicken Ovalbumin Upstream Promoter-Transcription Factor II (COUP-TFII) plays a role in Leydig cell steriodogenesis that is perturbed by dibutyl phthalate in rats but not in mice (Driesche, et al., 2012).

Finally, in an article published in Toxicological Sciences, Kamin Johnson, Nick Heger and Kim Boekelheide examined studies describing effects on the fetal male reproductive system, particularly antisteroidogenic-mediated effects (e.g., retained areolae and reduced AGD). These researchers have concluded that the evidence supports species-specific differences in testicular response following in utero exposure and that the rat, as opposed to the mouse or human, is the sensitive species to phthalate-induced effects. They also state that the data suggest that human fetal testis responds to phthalate exposure (in utero) like the mouse and not the rat.

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[4] Number of male pups calculated as 5 times the number of litters at PND 0, from Table 3 of Tyl et al., plus the number of males culled at PND 4, from page 250 of Tyl et al.

Overall then, these independent scientists – who are among the leaders in the field of male reproductive toxicity and who have focused on investigating a possible environmental etiology for male reproductive disorders – have produced compelling evidence for species-specific responses to phthalates with respect to male reproductive system effects following in utero exposures.[5]

These findings provide powerful support for the more-than-conservative approach afforded by a NOEL of 50 mg/kg/day, which would result in a MADL of 2900 mg/kg/day.

CONCLUSION

We thank OEHHA for undertaking promulgation of a MADL for BBP. For the reasons presented above, Ferro submits that 50 mg/kg/day provides a very conservative basis for setting a MADL for BBP. We accordingly ask that OEHHA select a NOEL of 50 mg/kg/day, which would result in a MADL of 2900 mg/kg/day.

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[5] We are aware of human studies that report associations between phthalate metabolites in the blood of women during pregnancy and developmental effects in their male offspring, including one study that found an association for monobenzyl phthalate and effects, while others did not. As acknowledged by OEHHA (OEHHA, *supra*, note 2, p. 4 of 12), the results of these studies are inconsistent and hard to interpret because of exposure to multiple phthalates. They most certainly do not provide a basis for finding that BBP causes male reproductive effects.

Table 1. Incidence of Reproductive Effects at Low and Mid-Doses

Study	Dose mg/kg/day	No. Male Pups	No. with retained areolae	AGD Statistically Reduced?
Tyl et al., 2004	0	384*	9	--
Nagao et al. 2000	20	125	--	No
Sumner et al. 2009	25	16	7	(No)**
Tyl et al. 2004	50	374*	0	No
Nagao et al. 2000	100	181	--	No
Tyl et al. 2004	250	368*	11 or 12	Yes

* Numbers calculated as described in note 4. F1 and F2 numbers combined.

** 2 of 16 male pups versus 0 of 17 male pups in control. For the reasons described in the text, Ferro does not believe statistical treatment of the data is appropriate; however, we note that OEHHA did not report statistical significance for these effects from its calculations (OEHHA, *supra* note 2).

Table 2. Summary of Reproductive/Developmental Toxicity Studies Considered in Deriving the BBP MADL

Study	Doses (mg/kg/day)	No. Litters per dose	No. Male pups per dose	LOAEL & effect	NOEL/ NOAEL	Comment
Nagao et al. (2000) guideline 2-gen repro	0, 20, 100, 500	25 per sex	160, 125, 181, 170	100 reduced birth wt in F1 only, PND 0 only	20/ 100	Effect at 100 appears to be artifact of no. pups born per litter; no effect in F2 pups
Tyl et al. (2004) guideline 2-gen repro	0, 50, 250, 750	30 per sex	384, 374, 368, 279	250 reduced AGD	50/ 250	Reproductive function, development and structure not impaired at 250
Sumner et al. (2009) mechanistic study	0, 25, 750	3 per sex	17, 16, 6	25 retained areolae, PND 11 but not PND 26	--/ 25	Reversible; statistically significant only on pup basis, not on litter basis

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